



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b>  <b>C07K 14/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 99/12964</b>  <b>(43) International Publication Date:</b> 18 March 1999 (18.03.99)
<b>(21) International Application Number:</b> PCT/US98/19037  <b>(22) International Filing Date:</b> 11 September 1998 (11.09.98)  <b>(30) Priority Data:</b> 60/058,786                      12 September 1997 (12.09.97)      US  <b>(71) Applicant (for all designated States except US):</b> BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> TSCHOPP, Jurg [CH/CH]; 10, chemin des Fontannins, CH-1066 Epalinges (CH).  <b>(74) Agent:</b> FLYNN, Kerry; Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> KAY – A NOVEL IMMUNE SYSTEM PROTEIN  <b>(57) Abstract</b>  Kay–ligand, a novel member of the tumor necrosis factor family (TNF), modified Kay–ligands and pharmaceutical compositions comprising them.		

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## **KAY- A NOVEL IMMUNE SYSTEM PROTEIN**

### **BACKGROUND OF THE INVENTION**

The present invention relates to a novel ligand, Kay, which is a member of the Tumor Necrosis Factor Family. This protein or its receptor may have anti-cancer  
5 and/or immunoregulatory applications. Furthermore, cells transfected with the genes for this novel ligand may be used in gene therapy to treat tumors, autoimmune and inflammatory diseases or inherited genetic disorders, and blocking antibodies to these proteins can have immunoregulatory applications.

### **BACKGROUND OF THE INVENTION**

10 The tumor-necrosis factor (TNF)-related cytokines are mediators of host defense and immune regulation. Members of this family exist in membrane-anchored forms, acting locally through cell-to-cell contact, or as secreted proteins capable of diffusing to more distant targets. A parallel family of receptors signals the presence of these molecules leading to the initiation of cell death or cellular proliferation and  
15 differentiation in the target tissue. Presently, the TNF family of ligands and receptors has at least 11 recognized receptor-ligand pairs, including: TNF:TNF-R; LT- $\alpha$ :TNF-R; LT- $\alpha/\beta$ :LT- $\beta$ -R; FasL:Fas; CD40L:CD40; CD30L:CD30; CD27L:CD27; OX40L:OX40 and 4-1BBL:4-1BB. The DNA sequences encoding these ligands have only about 25% to about 30% identity in even the most related cases, although the  
20 amino acid relatedness is about 50%.

The defining feature of this family of cytokine receptors is found in the cysteine rich extracellular domain initially revealed by the molecular cloning of two distinct TNF receptors.<sup>i</sup> This family of genes encodes glycoproteins characteristic of Type I transmembrane proteins with an extracellular ligand binding domain, a single  
25 membrane spanning region and a cytoplasmic region involved in activating cellular functions. The cysteine-rich ligand binding region exhibits a tightly knit disulfide linked core domain, which, depending upon the particular family member, is repeated multiple times. Most receptors have four domains, although there may be as few as three, or as many as six.

30 Proteins in the TNF family of ligands are characterized by a short N-terminal stretch of normally short hydrophilic amino acids, often containing several lysine or arginine residues thought to serve as stop transfer sequences. Next follows a

-2-

transmembrane region and an extracellular region of variable length, that separates the C-terminal receptor binding domain from the membrane. This region is sometimes referred to as the "stalk". The C-terminal binding region comprises the bulk of the protein, and often, but not always, contains glycosylation sites. These genes lack the classic signal sequences characteristic of type I membrane proteins, type II membrane proteins with the C terminus lying outside the cell, and a short N-terminal domain residing in the cytoplasm. In some cases, e.g., TNF and LT- $\alpha$ , cleavage in the stalk region can occur early during protein processing and the ligand is then found primarily in secreted form. Most ligands, however, exist in a membrane form, mediating localized signaling.

The structure of these ligands has been well-defined by crystallographic analyses of TNF, LT- $\alpha$ , and CD40L. TNF and lymphotoxin- $\alpha$  (LT- $\alpha$ ) are both structured into a sandwich of two anti-parallel  $\beta$ -pleated sheets with the "jelly roll" or Greek key topology.<sup>ii</sup> The rms deviation between the C $\alpha$  and  $\beta$  residues is 0.61 Å, suggesting a high degree of similarity in their molecular topography. A structural feature emerging from molecular studies of CD40L, TNF and LT- $\alpha$  is the propensity to assemble into oligomeric complexes. Intrinsic to the oligomeric structure is the formation of the receptor binding site at the junction between the neighboring subunits creating a multivalent ligand. The quaternary structures of TNF, CD40L and LT- $\alpha$  have been shown to exist as trimers by analysis of their crystal structures. Many of the amino acids conserved between the different ligands are in stretches of the scaffold  $\beta$ -sheet. It is likely that the basic sandwich structure is preserved in all of these molecules, since portions of these scaffold sequences are conserved across the various family members. The quaternary structure may also be maintained since the subunit conformation is likely to remain similar.

TNF family members can best be described as master switches in the immune system controlling both cell survival and differentiation. Only TNF and LT $\alpha$  are currently recognized as secreted cytokines contrasting with the other predominantly membrane anchored members of the TNF family. While a membrane form of TNF has been well-characterized and is likely to have unique biological roles, secreted TNF functions as a general alarm signaling to cells more distant from the site of the triggering event. Thus TNF secretion can amplify an event leading to the well-described changes in the vasculature lining and the inflammatory state of cells. In

-3-

contrast, the membrane bound members of the family send signals through the TNF type receptors only to cells in direct contact. For example T cells provide CD40 mediated “help” only to those B cells brought into direct contact via cognate TCR interactions. Similar cell-cell contact limitations on the ability to induce cell death apply to the well-studied Fas system.

It appears that one can segregate the TNF ligands into three groups based on their ability to induce cell death (Table III). First, TNF, Fas ligand and TRAIL can efficiently induce cell death in many lines and their receptors mostly likely have good canonical death domains. Presumably the ligand to DR-3 (TRAMP/WSL-1) would also all into this category. Next there are those ligands which trigger a weaker death signal limited to few cell types and TWEAK, CD30 ligand and LTa1b2 are examples of this class. How this group can trigger cell death in the absence of a canonical death domain is an interesting question and suggests that a separate weaker death signaling mechanism exists. Lastly, there are those members that cannot efficiently deliver a death signal. Probably all groups can have antiproliferative effects on some cell types consequent to inducing cell differentiation e.g. CD40 (Funakoshi et al., 1994)

The TNF family has grown dramatically in recent years to encompass at least 11 different signaling pathways involving regulation of the immune system. The widespread expression patterns of TWEAK and TRAIL indicate that there is still more functional variety to be uncovered in this family. This aspect has been especially highlighted recently in the discovery of two receptors that affect the ability of rous sarcoma and herpes simplex virus to replicate as well as the historical observations that TNF has anti-viral activity and pox viruses encode for decoy TNF receptors (Brojatsch et al., 1996; Montgomery et al., 1996; Smith, 1994; Vassalli, 1992).

TNF is a mediator of septic shock and cachexia<sup>iii</sup>, and is involved in the regulation of hematopoietic cell development.<sup>iv</sup> It appears to play a major role as a mediator of inflammation and defense against bacterial, viral and parasitic infections<sup>v</sup> as well as having antitumor activity.<sup>vi</sup> TNF is also involved in different autoimmune diseases.<sup>vii</sup> TNF may be produced by several types of cells, including macrophages, fibroblasts, T cells and natural killer cells.<sup>viii</sup> TNF binds to two different receptors, each acting through specific intracellular signaling molecules, thus resulting in different effects of TNF.<sup>ix</sup> TNF can exist either as a membrane bound form or as a soluble secreted cytokine.<sup>x</sup>

-4-

LT- $\alpha$  shares many activities with TNF, i.e. binding to the TNF receptors,<sup>xi</sup> but unlike TNF, appears to be secreted primarily by activated T cells and some  $\beta$ -lymphoblastoid tumors.<sup>xii</sup> The heteromeric complex of LT- $\alpha$  and LT- $\beta$  is a membrane bound complex which binds to the LT- $\beta$  receptor.<sup>xiii</sup> The LT system (LTs and LT-R) appears to be involved in the development of peripheral lymphoid organs since genetic disruption of LT- $\beta$  leads to disorganization of T and B cells in the spleen and an absence of lymph nodes.<sup>xiv</sup> The LT- $\beta$  system is also involved in cell death of some adenocarcinoma cell lines.<sup>xv</sup>

Fas-L, another member of the TNF family, is expressed predominantly on activated T cells.<sup>xvi</sup> It induces the death of cells bearing its receptor, including tumor cells and HIV-infected cells, by a mechanism known as programmed cell death or apoptosis.<sup>xvii</sup> Furthermore, deficiencies in either Fas or Fas-L may lead to lymphoproliferative disorders, confirming the role of the Fas system in the regulation of immune responses.<sup>xviii</sup> The Fas system is also involved in liver damage resulting from hepatitis chronic infection<sup>xix</sup> and in autoimmunity in HIV-infected patients.<sup>xx</sup> The Fas system is also involved in T-cell destruction in HIV patients.<sup>xxi</sup> TRAIL, another member of this family, also seems to be involved in the death of a wide variety of transformed cell lines of diverse origin.<sup>xxii</sup>

CD40-L, another member of the TNF family, is expressed on T cells and induces the regulation of CD40-bearing B cells.<sup>xxiii</sup> Furthermore, alterations in the CD40-L gene result in a disease known as X-linked hyper-IgM syndrome.<sup>xxiv</sup> The CD40 system is also involved in different autoimmune diseases<sup>xxv</sup> and CD40-L is known to have antiviral properties.<sup>xxvi</sup> Although the CD40 system is involved in the rescue of apoptotic B cells,<sup>xxvii</sup> in non-immune cells it induces apoptosis<sup>xxviii</sup>. Many additional lymphocyte members of the TNF family are also involved in costimulation.<sup>xxix</sup>

Generally, the members of the TNF family have fundamental regulatory roles in controlling the immune system and activating acute host defense systems. Given the current progress in manipulating members of the TNF family for therapeutic benefit, it is likely that members of this family may provide unique means to control disease. Some of the ligands of this family can directly induce the apoptotic death of many transformed cells e.g. LT, TNF, Fas ligand and TRAIL (Nagata, 1997). Fas and possibly TNF and CD30 receptor activation can induce cell death in nontransformed

-5-

lymphocytes which may play an immunoregulatory function (Amakawa et al., 1996; Nagata, 1997; Sytwu et al., 1996; Zheng et al., 1995). In general, death is triggered following the aggregation of death domains which reside on the cytoplasmic side of the TNF receptors. The death domain orchestrates the assembly of various signal transduction components which result in the activation of the caspase cascade (Nagata, 1997). Some receptors lack canonical death domains, e.g. LT $\beta$  receptor and CD30 (Browning et al., 1996; Lee et al., 1996) yet can induce cell death, albeit more weakly. It is likely that these receptors function primarily to induce cell differentiation and the death is an aberrant consequence in some transformed cell lines, although this picture is unclear as studies on the CD30 null mouse suggest a death role in negative selection in the thymus (Amakawa et al., 1996). Conversely, signaling through other pathways such as CD40 is required to maintain cell survival. Thus, there is a need to identify and characterize additional molecules which are members of the TNF family thereby providing additional means of controlling disease and manipulating the immune system.

#### SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a novel polypeptide referred to as Kay-ligand, which substantially obviates one or more of the problems due to the limitations and disadvantages of the related art. The inventors have discovered new members of the TNF family of cytokines, and defined both the human amino acid sequence of the protein, as well as the DNA sequences encoding these proteins. The claimed invention may be used to identify new diagnostics and therapeutics for numerous diseases and conditions as discussed in more detail below, as well as to obtain information about, and manipulate, the immune system and its processes. Additionally, the invention may be involved in the induction of cell death in carcinomas.

Additional features and advantages of the invention will be set forth in the description which follows, and in part will be apparent from the description, or may be learned by practice of the invention. The objectives and other advantages of the invention will be realized and attained by the compositions and methods particularly pointed out in the written description and claims hereof, as well as in the appended drawings.

-6-

Thus, to achieve these and other advantages, and in accordance with the purpose of the invention, as embodied and broadly described herein, the invention includes DNA sequences encoding Kay-ligand. Specifically, the invention relates to DNA sequences which encode the human Kay-ligand, SEQ. ID. NO.: 1. Additionally, the  
5 claimed invention relates to the amino acid sequence of this novel ligand. The amino acid sequence of human Kay-ligand is set forth in SEQ. ID. NO.: 2. Applicants have additionally provided in part the DNA sequence for murine Kay-ligand, SEQ. ID. NO.: 3, and the protein encoded by SEQ. ID. NO. 3 is provided in SEQ. ID. NO.: 4. In other  
10 embodiments, the invention relates to sequences that have at least 50% homology with DNA sequences encoding the C terminal receptor binding domain of the ligand and hybridize to the claimed DNA sequences or fragments thereof, and which encode the Kay- ligand having the sequences identified in SEQ. ID. NO. 1 or SEQ. ID. NO. 4.

The invention in certain embodiments furthermore relates to DNA sequences encoding Kay-ligand where the sequences are operatively linked to an expression  
15 control sequence. Any suitable expression control sequences are useful in the claimed invention, and can easily be selected by one skilled in the art.

The invention also contemplates recombinant DNAs comprising a sequence encoding Kay-ligand or fragments thereof, as well as hosts with stably integrated Kay-ligand sequences introduced into their genome, or possessing episomal elements. Any  
20 suitable host may be used in the invention, and can easily be selected by one skilled in the art without undue experimentation.

In other embodiments, the invention relates to methods of producing substantially pure Kay-ligands comprising the step of culturing transformed hosts. In yet other embodiments, the invention relates to the Kay ligand essentially free of  
25 normally associated animal proteins.

The invention encompasses Kay-ligand having the amino acid sequence identified in SEQ. ID. NO. 2. as well as fragments or homologs thereof. In various embodiments, the amino acid and/or the DNA sequences may comprise conservative insertions, deletions and substitutions, as further defined below or may comprise  
30 fragments of said sequences.

The invention relates in other embodiments to soluble constructs comprising Kay-ligand which may be used to directly trigger Kay-ligand mediated pharmacological events. Such events may have useful therapeutic benefits in the



-7-

treatment of cancer, tumors or the manipulation of the immune system to treat immunologic diseases. Soluble forms of the claimed ligands could be genetically reengineered to incorporate an easily recognizable tag, thereby facilitating the identification of the receptors for these ligands.

5           Additionally, in other embodiments the claimed invention relates to antibodies directed against the Kay-ligand, which can be used, for example, for the treatment of cancers, and manipulation of the immune system to treat immunologic disease.

          In yet other embodiments the invention relates to methods of gene therapy using the genes for Kay-ligand, as disclosed and claimed herein.

10           The pharmaceutical preparations of the invention may, optionally, include pharmaceutically acceptable carriers, adjuvants, fillers, or other pharmaceutical compositions, and may be administered in any of the numerous forms or routes known in the art.

          It is to be understood that both the foregoing general description and the  
15   following detailed description are exemplary and explanatory, and are intended to provide further explanation of the invention as claimed.

          The accompanying drawings are included to provide a further understanding of the invention, and are incorporated in, and constitute a part of this specification, illustrate several embodiments of the invention, and together with the description serve  
20   to explain the principles of the invention.

#### DESCRIPTION OF THE DRAWINGS

Figure 1: An alignment of the amino acid sequences of murine and human Kay Ligand. The murine sequence in the upper line was obtained by direct cloning of the cDNA.  
25   The human sequence reflects a composite of a partial cDNA sequence and 5' RACE determination. The third, bottom sequence lines shows the consensus sequence.

Figure 2: A fragment of human KayL cDNA was used to probe a northern blot of RNA's from various human tissues. It can be seen that a roughly 2.4 kb KayL RNA is  
30   expressed primarily in the spleen and peripheral blood lymphocytes, i.e. in the secondary lymphoid organs.

#### DETAILED DESCRIPTION

-8-

Reference will now be made in detail to the present preferred embodiments of the invention. This invention relates to DNA sequences that code for human or mouse Kay-ligands, fragments and homologs thereof, and expression of those DNA sequences in hosts transformed with them. The invention relates to uses of these DNA sequences and the peptides encoded by them. Additionally, the invention encompasses both human and mouse amino acid sequences for Kay-ligand or fragments thereof, as well as pharmaceutical compositions comprising or derived from them.

#### A. DEFINITIONS

"Homologous", as used herein, refers to the sequence similarity between sequences of molecules being compared. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

A "purified preparation" or a "substantially pure preparation" of a polypeptide, as used herein, means a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from other substances, e.g., antibodies, matrices, etc., which are used to purify it.

"Transformed host" as used herein is meant to encompass any host with stably integrated sequence, i.e. Kay-ligand sequence, introduced into its genome or a host possessing sequence, i.e. Ligand encoding episomal elements.

A "treatment", as used herein, includes any therapeutic treatment, e.g., the administration of a therapeutic agent or substance, e.g., a drug.

A "substantially pure nucleic acid", e.g., a substantially pure DNA, is a nucleic acid which is one or both of: (1) not immediately contiguous with either one or both of the sequences, e.g., coding sequences, with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the

-9-

organism from which the nucleic acid is derived; or (2) which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the  
5 genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Substantially pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding Kay-ligand.

10 The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

"Biologically active" as used herein, means having an in vivo or in vitro activity which may be performed directly or indirectly. Biologically active fragments of Kay ligand may have, for example, 70% amino acid homology with the active site of the  
15 Ligands, more preferably at least 80%, and most preferably, at least 90% homology. Identity or homology with respect to the Ligands is defined herein as the percentage of amino acid residues in the candidate sequence which are identical to the Kay-ligand residues in SEQ. ID. NO. 2.

The practice of the present invention will employ, unless otherwise indicated,  
20 conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature.

-10-

**B. DNA SEQUENCES OF THE INVENTION**

As described herein, one aspect of the invention features a substantially pure (or recombinant) nucleic acid which includes a nucleotide sequence encoding a Kay-ligand, such as the DNA described in SEQ. ID. NO. 1 and/or equivalents of such nucleic acids.

5 The term nucleic acid as used herein can include fragments and equivalents, such as, for example, sequences encoding functionally equivalent peptides. Equivalent nucleotide sequences may include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants, mutations, etc. and include sequences that differ from the nucleotide sequence encoding Kay-ligand shown in SEQ. ID NO: 1,  
10 due to the degeneracy of the genetic code.

The inventor describes herein the human and sequences; the invention will be described generally by reference to the human sequences, although one skilled in the art will understand that the mouse sequences are encompassed herein. The human proteins appear to have all of the characteristics of the TNF family, i.e., a type II membrane  
15 protein organization and conservation of the sequence motifs involved in the folding of the protein into the TNF anti-parallel  $\beta$ -sheet structure.

The nucleotide sequence for Kay-ligand is set forth in SEQ. ID. NO. 1; the amino acid sequence for Kay-ligand is described in SEQ. ID. NO. 2.

The sequences of the invention can be used to prepare a series of DNA probes  
20 that are useful in screening various collections of natural and synthetic DNAs for the presence of DNA sequences that are closely related to Kay-ligand, or fragments or derivatives thereof. One skilled in the art will recognize that reference to Kay-ligand as used herein, refers also to biologically active derivatives, fragments or homologs thereof.

25 The DNA sequences encoding the Kay-Ligand of the invention can be employed to produce the claimed peptides on expression in various prokaryotic and eukaryotic hosts transformed with them. These peptides may be used in anti-cancer, and immunoregulatory applications. In general, this comprises the steps of culturing a host transformed with a DNA molecule containing the sequence encoding Kay-ligand,  
30 operatively-linked to an expression control sequence.

The DNA sequences and recombinant DNA molecules of the present invention can be expressed using a wide variety of host/vector combinations. For example, useful vectors may consist of segments of chromosomal, non-chromosomal or synthetic DNA

-11-

sequences. The expression vectors of the invention are characterized by at least one expression control sequence that may be operatively linked to the Kay-Ligand DNA sequence inserted in the vector, in order to control and to regulate the expression of the DNA sequence.

5           Furthermore, within each expression vector, various sites may be selected for insertion of the Kay-Ligand sequence of the invention. The sites are usually designated by a restriction endonuclease which cuts them, and these sites and endonucleases are well recognized by those skilled in the art. It is of course to be understood that an expression vector useful in this invention need not have a restriction endonuclease site  
10   for insertion of the desired DNA fragment. Instead, the vector may be cloned to the fragment by alternate means. The expression vector, and in particular the site chosen therein for insertion of a selected DNA fragment, and its operative linking therein to an expression control sequence, is determined by a variety of factors. These factors include, but are not limited to, the size of the protein to be expressed, the susceptibility  
15   of the desired protein to proteolytic degradation by host cell enzymes, number of sites susceptible to a particular restriction enzyme, contamination or binding of the protein to be expressed by host cell proteins which may prove difficult to remove during purification. Additional factors which may be considered include expression characteristics such as the location of start and stop codons relative to the vector  
20   sequences, and other factors which will be recognized by those skilled in the art. The choice of a vector and insertion site for the claimed DNA sequences is determined by a balancing of these factors, not all selections being equally effective for a desired application. However, it is routine for one skilled in the art to analyze these parameters and choose an appropriate system depending on the particular application.

25           One skilled in the art can readily make appropriate modifications to the expression control sequences to obtain higher levels of protein expression, i.e. by substitution of codons, or selecting codons for particular amino acids that are preferentially used by particular organisms, to minimize proteolysis or to alter glycosylation composition. Likewise, cysteines may be changed to other amino acids to  
30   simplify production, refolding or stability problems.

Thus, not all host/expression vector combinations function with equal efficiency in expressing the DNA sequences of this invention. However, a particular selection of a host/expression vector combination may be made by those of skill in the art. Factors

-12-

one may consider include, for example, the compatibility of the host and vector, toxicity to the host of the proteins encoded by the DNA sequence, ease of recovery of the desired protein, expression characteristics of the DNA sequences and expression control sequences operatively linked to them, biosafety, costs and the folding, form or other necessary post-expression modifications of the desired protein.

The Kay-ligand and homologs thereof produced by hosts transformed with the sequences of the invention, as well as native Kay-ligand purified by the processes of this invention, or produced from the claimed amino acid sequences, are useful in a variety of compositions and methods for anticancer, antitumor and immunoregulatory applications. They are also useful in therapy and methods directed to other diseases.

This invention also relates to the use of the DNA sequences disclosed herein to express this ligand under abnormal conditions, i.e. in a gene therapy setting. Kay-ligand may be expressed in tumor cells under the direction of promoters appropriate for such applications. Such expression could enhance anti-tumor immune responses or directly affect the survival of the tumor. The claimed ligand can also affect the survival of an organ graft by altering the local immune response. In this case, the graft itself or the surrounding cells would be modified with an engineered gene encoding Kay-ligand.

Another aspect of the invention relates to the use of the isolated nucleic acid encoding the Kay-ligand in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize under cellular conditions with the cellular mRNA and/or DNA encoding the ligand of interest, so as to inhibit expression of the encoded protein, i.e. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to a range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention can be delivered, for example, as an expression plasmid, which, when transcribed in the cell, produces RNA which is complementary to at least a portion of the cellular mRNA which encodes Kay-ligand. Alternatively, the antisense construct can be an oligonucleotide probe which is generated *ex vivo*. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, and are therefore stable in

-13-

vivo. Exemplary nucleic acids molecules for use as antisense oligonucleotides are phosphoramidates, phosphothioate and methylphosphonate analogs of DNA (See, e.g., 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by Van Der Krol et al., (1988) *Biotechniques* 6:958-976; and Stein et al. (1988) *Cancer Res* 48: 2659-2668, specifically incorporated herein by reference.

### C. KAY-LIGAND AND AMINO ACID SEQUENCES THEREFOR

10           The Kay-ligand of the invention, as discussed above, is a member of the TNF family. The protein, fragments or homologs thereof may have wide therapeutic and diagnostic applications.

          The Kay-ligand is present primarily in the spleen and in peripheral blood lymphocytes, strongly indicating a regulatory role in the immune system. Comparison  
15       of the claimed Kay-ligand sequences with other members of the human TNF family reveals considerable structural similarity. All the proteins share several regions of sequence conservation in the extracellular domain.

          Although the precise three-dimensional structure of the claimed ligand is not known, it is predicted that, as a member of the TNF family, it may share certain  
20       structural characteristics with other members of the family.

          The novel polypeptides of the invention specifically interact with a receptor, which has not yet been identified. However, the peptides and methods disclosed herein enable the identification of receptors which specifically interact with the claimed Kay-ligand or fragments thereof.

25           The claimed invention in certain embodiments includes peptides derived from Kay-ligand which have the ability to bind to their receptors. Fragments of the Kay-ligands can be produced in several ways, e.g., recombinantly, by PCR, proteolytic digestion or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end or both ends of a  
30       nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments.

-14-

Polypeptide fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f- moc or t-boc chemistry. For example, peptides and DNA sequences of the present invention may be arbitrarily divided into fragments of desired length with no overlap of the fragment, or divided  
5 into overlapping fragments of a desired length. Methods such as these are described in more detail below.

#### D. Generation of Soluble Forms of Kay-ligand and Tumor-ligand

Soluble forms of the Kay-ligand can often signal effectively and hence can be administered as a drug which now mimics the natural membrane form. It is possible  
10 that the Kay-ligand claimed herein are naturally secreted as soluble cytokines, however, if not, one can reengineer the gene to force secretion. To create a soluble secreted form of Kay-ligand, one would remove at the DNA level the N-terminus transmembrane regions, and some portion of the stalk region, and replace them with a type I leader or alternatively a type II leader sequence that will allow efficient proteolytic cleavage in  
15 the chosen expression system. A skilled artisan could vary the amount of the stalk region retained in the secretion expression construct to optimize both receptor binding properties and secretion efficiency. For example, the constructs containing all possible stalk lengths, i.e. N-terminal truncations, could be prepared such that proteins starting at amino acids 81 to 139 would result. The optimal length stalk sequence would result  
20 from this type of analysis.

#### E. Generation of Antibodies Reactive with the Kay-ligand

The invention also includes antibodies specifically reactive with the claimed Kay-ligand or its receptors. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory*  
25 *Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers, or other techniques, well known in the art.

An immunogenic portion of the claimed Kay-ligand or its receptors can be  
30 administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other



-15-

immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of Kay-ligand or its receptors, e.g. antigenic determinants of a polypeptide of SEQ. ID. NO.: 2, or a closely related human or non-human mammalian homolog (e.g. 70, 80 or 90 percent homologous, more preferably at least 95 percent homologous). In yet a further preferred embodiment of the present invention, the anti-Kay-ligand or anti-Kay-ligand-receptor antibodies do not substantially cross react (i.e. react specifically) with a protein which is e.g., less than 80 percent homologous to SEQ. ID. NO. 2 or 6; preferably less than 90 percent homologous with SEQ. ID. NO.: 2; and, most preferably less than 95 percent homologous with SEQ. ID. NO.:2. By "not substantially cross react", it is meant that the antibody has a binding affinity for a non-homologous protein which is less than 10 percent, more preferably less than 5 percent, and even more preferably less than 1 percent, of the binding affinity for a protein of SEQ. ID. NO. 2.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with Kay-ligand, or its receptors. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example,  $F(ab')_2$  fragments can be generated by treating antibody with pepsin. The resulting  $F(ab')_2$  fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibodies of the present invention are further intended to include biospecific and chimeric molecules having anti-Kay-ligand or anti-Kay-ligand -receptor activity. Thus, both monoclonal and polyclonal antibodies (Ab) directed against Kay-ligand, Tumor-ligand and their receptors, and antibody fragments such as Fab' and  $F(ab')_2$ , can be used to block the action of the Ligand and their respective receptor.

Various forms of antibodies can also be made using standard recombinant DNA techniques. (Winter and Milstein, Nature 349: 293-299 (1991) specifically incorporated by reference herein.) For example, chimeric antibodies can be constructed in which the antigen binding domain from an animal antibody is linked to a human constant domain (e.g. Cabilly et al., U.S. 4,816,567, incorporated herein by reference). Chimeric antibodies may reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

-16-

In addition, recombinant “humanized antibodies” which recognize Kay-ligand or its receptors can be synthesized. Humanized antibodies are chimeras comprising mostly human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. Animals are immunized with the desired antigen, the  
5 corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (i.e. inter species) sequences in human antibodies,  
10 and thus are less likely to elicit immune responses in the treated subject.

Construction of different classes of recombinant antibodies can also be accomplished by making chimeric or humanized antibodies comprising variable domains and human constant domains (CH1, CH2, CH3) isolated from different classes of immunoglobulins. For example, antibodies with increased antigen binding site  
15 valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human : chain constant regions. (Arulanandam et al., J. Exp. Med., 177: 1439-1450 (1993), incorporated herein by reference.)

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid  
20 residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling. (Queen et al., Proc. Natl. Acad. Sci. 86: 10029-33 (1989) incorporated herein by reference.

#### F. Generation of Analogs: Production of Altered DNA and Peptide Sequences

25 Analogs of the claimed Kay-ligand can differ from the naturally occurring Kay-ligand in amino acid sequence, or in ways that do not involve sequence, or both. Non-sequence modifications include in vivo or in vitro chemical derivatization of the Kay-ligand. Non-sequence modifications include, but are not limited to, changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

30 Preferred analogs include Kay-ligand biologically active fragments thereof, whose sequences differ from the sequence given in SEQ. ID NO. 2, by one or more conservative amino acid substitutions, or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the activity of Kay-ligand.

-17-

Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g. substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and, phenylalanine, 5 tyrosine.

-18-

TABLE 1

## CONSERVATIVE AMINO ACID REPLACEMENTS

for amino Acid	code	replace with any of:
Alanine	A	D-Ala, Gly, Beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, -Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met

-19-

Lysine	K	D-Lys, Arg, D-Arg, Homo-arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3, 4 or 5-phenylproline, cis-3, 4, or 5-phenylproline
Proline	P	D-Pro, L-I-thoazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Useful methods for mutagenesis include PCR mutagenesis and saturation mutagenesis as discussed in more detail below. A library of random amino acid

-20-

sequence variants can also be generated by the synthesis of a set of degenerate oligonucleotide sequences.

#### -PCR Mutagenesis

In PCR mutagenesis, reduced Taq polymerase fidelity can be used to introduce random mutations into a cloned fragment of DNA (Leung et al., 1989, *Technique* 1:11-15). This is a very powerful and relatively rapid method of introducing random mutations. The DNA region to be mutagenized can be amplified using the polymerase chain reaction (PCR) under conditions that reduce the fidelity of DNA synthesis by Taq DNA polymerase, e.g., by using a dGTP/dATP ratio of five and adding  $Mn^{2+}$  to the PCR reaction. The pool of amplified DNA fragments can be inserted into appropriate cloning vectors to provide random mutant libraries.

#### -Saturation Mutagenesis

Saturation mutagenesis allows for the rapid introduction of a large number of single base substitutions into cloned DNA fragments (Mayers et al., 1985, *Science* 229:242). This technique includes generation of mutations, e.g., by chemical treatment or irradiation of single-stranded DNA *in vitro*, and synthesis of a complimentary DNA strand. The mutation frequency can be modulated by modulating the severity of the treatment, and essentially all possible base substitutions can be obtained. Because this procedure does not involve a genetic selection for mutant fragments both neutral substitutions, as well as of a protein can be prepared by random mutagenesis of DNA which those that alter function, can be obtained. The distribution of point mutations is not biased toward conserved sequence elements.

#### -Degenerate Oligonucleotides

A library of homologs can also be generated from a set of degenerate oligonucleotide sequences. Chemical synthesis of degenerate sequences can be carried out in an automatic DNA synthesizer, and the synthetic genes then ligated into an appropriate expression vector. The synthesis of degenerate oligonucleotides is known in the art<sup>xxx</sup>. Such techniques have been employed in the directed evolution of other proteins<sup>xxxi</sup>.

Non-random or directed, mutagenesis techniques can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein. The sites for mutation can be modified

-21-

individually or in series, e.g., by (1) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (2) deleting the target residue, or (3) inserting residues of the same or a different class adjacent to the located site, or combinations of options 1-3.

5           -Alanine Scanning Mutagenesis

Alanine scanning mutagenesis is a useful method for identification of certain residues or regions of the desired protein that are preferred locations or domains for mutagenesis, Cunningham and Wells (*Science* 244:1081-1085, 1989) specifically incorporated by reference. In alanine scanning, a residue or group of target residues are  
10 identified (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine). Replacement of an amino acid can affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions can then be refined by introducing further or  
15 other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed desired protein subunit variants are screened for the  
20 optimal combination of desired activity.

          -Oligonucleotide-Mediated Mutagenesis

Oligonucleotide-mediated mutagenesis is a useful method for preparing substitution, deletion, and insertion variants of DNA, see, e.g., Adelman et al., (*DNA* 2:183, 1983) incorporated herein by reference. Briefly, the desired DNA can be altered  
25 by hybridizing an oligonucleotide encoding a mutation to a DNA template, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the desired protein. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected  
30 alteration in the desired protein DNA. Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will

-22-

hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al. (*Proc. Natl. Acad. Sci. USA*, 75: 5765[1978]) incorporated herein by reference.

5           -Cassette Mutagenesis

Another method for preparing variants, cassette mutagenesis, is based on the technique described by Wells et al. (*Gene*, 34:315[1985]) incorporated herein by reference. The starting material can be a plasmid (or other vector) which includes the protein subunit DNA to be mutated. The codon(s) in the protein subunit DNA to be mutated are identified. There must be a unique restriction endonuclease site on each side of the identified mutation site(s). If no such restriction sites exist, they may be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the desired protein subunit DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are comparable with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated desired protein subunit DNA sequence.

          -Combinatorial Mutagenesis

Combinatorial mutagenesis can also be used to generate mutants. E.g., the amino acid sequences for a group of homologs or other related proteins are aligned, preferably to promote the highest homology possible. All of the amino acids which appear at a given position of the aligned sequences can be selected to create a degenerate set of combinatorial sequences. The variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level, and is encoded by a variegated gene library. For example, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential sequences are expressible as individual peptides, or alternatively, as a set of larger fusion proteins containing the set of degenerate sequences.



-23-

Various techniques are known in the art for screening generated mutant gene products. Techniques for screening large gene libraries often include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the genes under conditions in which  
5 detection of a desired activity, e.g., in this case, binding to Kay-ligand or its receptor, facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Each of the techniques described below is amenable to high through-put analysis for screening large numbers of sequences created, e.g., by random mutagenesis techniques.

10 The invention also provides for reduction of the protein binding domains of the claimed polypeptides or their receptors, to generate mimetics, e.g. peptide or non-peptide agents. The peptide mimetics are able to disrupt binding of Kay-ligand with its receptor. The critical residues of the Kay-ligand involved in molecular recognition of a receptor polypeptide or of a downstream intracellular protein, can be determined and  
15 used to generate the Kay-ligand or its receptor-derived peptidomimetics which competitively or noncompetitively inhibit binding of the Kay-ligand with a receptor. (see, for example, "Peptide inhibitors of human papilloma virus protein binding to retinoblastoma gene protein" European patent applications EP-412,762A and EP-B31,080A), specifically incorporated herein by reference.

#### 20 G. PHARMACEUTICAL COMPOSITIONS

By making available purified and recombinant- Kay-ligands, the present invention provides assays which can be used to screen for drug candidates which are either agonists or antagonists of the normal cellular function, in this case, of Kay-ligand, or its receptor. In one embodiment, the assay evaluates the ability of a  
25 compound to modulate binding between the Kay-ligand and their receptors. A variety of assay formats will suffice and, in light of the present inventions, will be comprehended by the skilled artisan.

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of  
30 compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is

-24-

mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with other proteins or change in enzymatic properties of the molecular target.

Pharmaceutical compositions of the invention may comprise a therapeutically effective amount of Kay-ligand, or its receptor, or fragments or mimetics thereof, and, optionally may include pharmaceutically acceptable carriers. Accordingly, this invention provides methods for treatment of cancer, and methods of stimulating, or in certain instances, inhibiting the immune system, or parts thereof by administering a pharmaceutically effective amount of a compound of the invention or its pharmaceutically acceptable salts or derivatives. It should of course be understood that the compositions and methods of this invention can be used in combination with other therapies for various treatments.

The compositions can be formulated for a variety of routes of administration, including systemic, topical or localized administration. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous for injection, the compositions of the invention can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compositions may be formulated in solid form and, optionally, redissolved or suspended immediately prior to use. Lyophilized forms are also included in the invention.

The compositions can be administered orally, or by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art, and include, for example, for transmucosal administration, bile salts, fusidic acid derivatives, and detergents. Transmucosal administration may be through nasal sprays or using suppositories. For oral administration, the compositions are formulated into conventional oral administration forms such as capsules, tablets, and tonics. For topical administration, the compositions of the invention are formulated into ointments, salves, gels, or creams as known in the art.

Preferably the compositions of the invention will be in the form of a unit dose and will be administered one or more times a day. The amount of active compound

-25-

administered at one time or over the course of treatment will depend on many factors. For example, the age and size of the subject, the severity and course of the disease being treated, the manner and form of administration, and the judgments of the treating physician. However, an effective dose may be in the range of from about 0.005 to  
5 about 5 mg/kg/day, preferably about 0.05 to about 0.5 mg/kg/day. One skilled in the art will recognize that lower and higher doses may also be useful.

Gene constructs according to the invention can also be used as a part of a gene therapy protocol to deliver nucleic acids encoding either an agonistic or antagonistic form of a Kay-ligand polypeptide.

10 Expression constructs of the claimed Kay-ligand can be administered in any biologically effective carrier, e.g., any formulation or composition capable of effectively delivering the gene for the claimed Kay-ligand to cells in vivo. Approaches include insertion of the gene in viral vectors which can transfect cells directly, or delivering plasmid DNA with the help of, for example, liposomes, or intracellular  
15 carriers, as well as direct injection of the gene construct. Viral vector transfer methods are preferred.

A pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the  
20 complete gene delivery system can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the target DNA, RNA or amino  
25 acid sequences to which they specifically bind. In other aspects, the claimed invention may be used to evaluate a chemical entity for its ability to interact with, e.g., bind or physically associate with the claimed Kay-ligand, or fragment thereof. The method includes contacting the chemical entity with the Kay-ligand, and evaluating the ability of the entity to interact with the Kay-ligand. Additionally, the Kay-ligand of the  
30 invention can be used in methods of evaluating naturally occurring Kay-ligand or receptors of the Kay-ligand, as well as to evaluate chemical entities which associate or bind with receptors of the Kay-ligand.

-26-

In certain aspects, the claimed invention features a method for evaluating a chemical entity for the ability to modulate the interaction between Kay-ligand and its receptor. The method includes combining a Kay-ligand receptor, and the Kay-ligand under conditions wherein the pair is capable of interacting, adding the chemical entity to be evaluated and detecting the formation or dissolution of complexes. These modulating agents may be further evaluated in vitro, e.g. by testing its activity in a cell free system, and then, optionally administering the compound to a cell or animal, and evaluating the effect.

#### H. EXAMPLES

- 10 c) Isolation of a receptor binding to the claimed Kay-ligand.

Ligands of the TNF family can be used to identify and clone receptors. With the described Kay-ligand sequences, one could fuse the 5' end of the extracellular domain of the Kay-ligand which constitutes the receptor binding sequence to a marker or tagging sequence and then add a leader sequence that will force secretion of the Kay-ligand in any of a number of expression systems. One example of this technology is described by Browning et al., (1996) (JBC 271, 8618-8626) where the LT- $\beta$  ligand was secreted in such a form. The VCAM leader sequence was coupled to a short myc peptide tag followed by the extracellular domain of the LT- $\beta$ . The VCAM sequence is used to force secretion of the normally membrane bound LT- $\beta$  molecule. The secreted protein retains a myc tag on the N-terminus which does not impair the ability to bind to a receptor. Such a secreted protein can be expressed in either transiently transfected Cos cells or a similar system, e.g., EBNA derived vectors, insect cell/baculovirus, picchia etc. The unpurified cell supernatant can be used as a source of the tagged ligand.

25 Cells expressing the receptor can be identified by exposing them to the tagged ligand. Cells with bound ligand are identified in a FACS experiment by labeling the myc tag with an anti-myc peptide antibody (9E10) followed by phycoerythrin (or a similar label) labeled anti-mouse immunoglobulin. FACS positive cells can be readily identified and would serve as a source of RNA encoding for the receptor. An expression library would then be prepared from this RNA via standard techniques and separated into pools. Pools of clones would be transfected into a suitable host cell and binding of the tagged ligand to receptor positive transfected cells determined via microscopic examination, following labeling of bound myc peptide tag with an enzyme

30

-27-

labeled anti-mouse Ig reagent, i.e. galactosidase, alkaline phosphatase or luciferase labeled antibody. Once a positive pool has been identified, the pool size would be reduced until the receptor encoding cDNA is identified. This procedure could be carried out with either the mouse or human Kay-ligand as one may more readily lead to

5 a receptor.

It will be apparent to those skilled in the art that various modifications and variations can be made in the novel Kay-ligand, compositions and methods of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this  
10 invention provided that they come within the scope of the appended claims and their equivalents.

-28-

I claim:

1. A DNA sequence encoding Kay-ligand or a fragment thereof.
2. A DNA sequence encoding Kay-ligand, said sequence consisting essentially of SEQ. ID. NO. 1 or SEQ. ID. NO. 3.
- 5 3. A DNA sequence consisting essentially of SEQ. ID. NO. 1 or SEQ. ID. NO. 3, said DNA encoding a polypeptide, said polypeptide consisting essentially of SEQ. ID. NO. 2 or SEQ. ID. NO. 4.
4. A DNA sequence that hybridizes to at least a fragment of SEQ. ID NO. 1 or SEQ. ID NO. 3 said fragment comprising at least 20 consecutive bases, said  
10 DNA sequence encoding a polypeptide that is at least 30% homologous with an active site of Kay-ligand.
5. A DNA sequence according to claim 2 wherein said sequence consists essentially of SEQ. ID. NO. 1 or SEQ. ID. NO. 3 with conservative substitutions, alterations or deletions.
- 15 6. A recombinant DNA molecule comprising a DNA sequence encoding Kay-ligand, said sequence operatively linked to an expression control sequence.
7. The molecule of claim 6 comprising SEQ. ID. NO. 1 or SEQ. ID. NO. 3.
8. A unicellular host transformed with a recombinant DNA molecule of claim 6 or 7.
- 20 9. A DNA sequence encoding Kay-ligand having the amino acid sequence of SEQ. ID. NO. 2 or SEQ. ID. NO 4.
10. A method for producing substantially pure Kay-ligand comprising the step of culturing the unicellular host of claim 8.
11. Kay-ligand essentially free of normally associated animal proteins.

-29-

12. The Kay-ligand of claim 11 consisting essentially of SEQ. ID. NO. 2 or SEQ. ID. NO. 4.
13. A pharmaceutical composition comprising a therapeutically effective amount of Kay-ligand or an active fragment thereof, and a pharmaceutically acceptable carrier.
14. A method for preventing or reducing the severity of an autoimmune disease comprising the step of administering a therapeutically effective amount of a pharmaceutical composition according to claim 13.
15. The pharmaceutical composition of claim 13 wherein said Kay-ligand or active fragment thereof comprises SEQ. ID. NO. 2 , or SEQ. ID. NO. 4, or a biologically active fragment thereof.
16. A method for preventing or reducing the severity of an immune response to a tissue graft comprising the step of administering a therapeutically effective amount of a pharmaceutical composition according to claim 13.
17. A method for stimulating the immune system comprising administering the composition of claim 13.
18. A method for suppressing the immune system comprising administering an effective amount of the pharmaceutical composition according to claim 13.
19. A method for treating cancer comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 13.
20. A method for identifying a receptor for the Kay-ligand comprising:
  - a. providing the Kay-ligand or a fragment thereof,
  - b. labeling said Kay-ligand or fragment thereof with a detectable label;
  - c. screening a composition to detect receptors which bind to the detectably labeled Kay-ligand of step b.

-30-

21. A soluble biologically active fragment of the Kay-ligand of claim 11.
22. A polypeptide comprising an amino acid sequence that is encoded by a DNA selected from the group consisting of:
- 5      a. a DNA sequence comprising SEQ. ID. NO. 1 or SEQ. ID. NO. 3;
- b. a DNA sequence that hybridizes to the DNA defined in a. and coding on expression for a polypeptide that is at least 40% homologous with the Kay-ligand of claim 12.
23. An antibody preparation that is reactive to Kay-ligand or its receptor or biologically active fragments thereof.
- 10    24. The antibody preparation of claim 23 comprising monoclonal antibodies.
25. A method for producing an antibody preparation reactive to Kay-ligand or its receptor comprising the step of immunizing an organism with Kay-ligand or its receptor, or an antigenic fragment thereof.
26. An antisense nucleic acid against Kay-ligand comprising a nucleic acid
- 15      sequence hybridizing to at least a portion of SEQ. ID. NO. 1 or SEQ. ID. NO. 3.
27. A pharmaceutical composition comprising an antibody preparation according to claim 24.
28. A method of expressing a gene in a mammalian cell comprising:
- a. introducing a gene encoding Kay-ligand into a cell;
- 20      b. allowing said cell to live under conditions such that said gene is expressed in said mammal.
29. A method of treating a disorder related to Kay-ligand in a mammal
- a. introducing into a cell a therapeutically effective amount of a vector comprising a gene encoding the Kay-ligand; and
- 25      b. expressing said gene in said mammalian cell.



-31-

30. The method of claim 29 wherein the mammal is a human.
31. The method of claim 29 wherein said vector is a virus.
32. A method of inducing cell death comprising the administration of an agent capable of interfering with the binding of Kay-ligand to a receptor.
- 5 33. The method of claim 32 further comprising the administration of interferon- $\gamma$ .
34. A method of treating, suppressing or altering an immune response involving a signaling pathway between Kay-ligand and its receptor, said method comprising the step of administering an effective amount of an agent capable of interfering with the association between Kay-ligand and its receptor.
- 10 35. The method of claim 34 wherein said immune response involves human adenocarcinoma cells.

## SEQ. ID. NO. 1

1 TGCCAAGCCC TGCCATGTAG TGCACGCAGG ACATCAACAA ACACAGATAA  
 51 CAGGAAATGA TCCATTCCCT GTGGTCACTT ATTCTAAAGG CCCC AACCTT  
 5 101 CAAAGTTCAA GTAGTGATAT GGATGACTCC ACAGAAAGGG AGCAGTCACG  
 151 CCTTACTTCT TGCCTTAAGA AAAGAGAAGA AATGAAACTG AAGGAGTGTG  
 201 TTTCCATCCT CCCACGGAAG GAAAGCCCCT CTGTCCGATC CTCCAAAGAC  
 251 GGAAAGCTGC TGGCTGCAAC CTTGCTGCTG GCACTGCTGT CTTGCTGCCT  
 301 CACGGTGGTG TCTTTCTACC AGGTGGCCGC CCTGCAAGGG GACCTGGCCA  
 10 351 GCCTCCGGGC AGAGCTGCAG GGCCACCACG CGGAGAAGCT GCCAGCAGGA  
 401 GCAGGAGCCC CCAAGGCCGG CCTGGAGGAA GCTCCAGCTG TCACCGCGGG  
 451 ACTGAAAATC TTTGAACCAC CAGCTCCAGG AGAAGGCAAC TCCAGTCAGA  
 501 ACAGCAGAAA TAAGCGTGCC GTTCAGGGTC CAGAAGAAAC AGTCACTCAA  
 551 GACTGCTTGC AACTGATTGC AGACAGTGAA ACACCAACTA TACAAAAAGG  
 15 601 ATCTTACACA TTTGTTCCAT GGCTTCTCAG CTTTAAAGG GGAAGTGCCC  
 651 TAGAAGAAAA AGAGAATAAA ATATTGGTCA AAGAACTGG TTACTTTTTT  
 701 ATATATGGTC AGGTTTTATA TACTGATAAG ACCTACGCCA TGGGACATCT  
 751 AATTCAGAGG AAGAAGGTCC ATGTCTTTGG GGATGAATTG AGTCTGGTGA  
 801 CTTTGTTTCG ATGTATTCAA AATATGCCTG AAACACTACC CAATAATTCC  
 20 851 TGCTATTTCAG CTGGCATTCG AAAACTGGAA GAAGGAGATG AACTCCAACCT  
 901 TGCAATACCA AGAGAAAATG CACAAATATC ACTGGATGGA GATGTCACAT  
 951 TTTTGGTGC ATTGAACTG CTGTGACCTA CTTACACCAT GTCTGTAGCT  
 1001 ATTTTCCTCC CTTTCTCTGT ACCTCTAAGA AGAAAGAATC TAACTGAAAA  
 1051 TA

## SEQ. ID NO. 2

1 MDDSTEREQS RLTSCLKKRE EMKLKECVSI LPRKESPSVR SSKDGKLLAA  
 51 TLLLALLSCC LTVVSFYQVA ALQGD LASLR AELQGHHA EK LPAGAGAPKA  
 101 GLEEAPAVTA GLKIFEPPAP GEGNSSQNSR NKRAVQGP EE TVTQDCLQLI  
 30 151 ADSEPTTIQK GSYTFVPWLL SFKRGSAL EE KENKILVK ET GYFFIYGQVL  
 201 YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL PNNSCYSAGI  
 251 AKLEEGDELQ LAIPRENAQI SLDDGVTF FG ALKLL

## SEQ. ID NO. 3

1 GTGGTCACTT ACTCCAAAGG CCTAGACCTT CAAAGTGCTC CTCGTGGAAT  
 51 GGATGAGTCT GCAAAGACCC TGCCACCACC GTGCCTCTGT TTTTGCTCCG  
 101 AGAAAGGAGA AGATATGAAA GTGGGATATG ATCCCATCAC TCCGCAGAAG  
 151 GAGGAGGGTG CCTGGTTTGG GATCTGCAGG GATGGAAGGC TGCTGGCTGC  
 201 TACCCTCCTG CTGGCCCTGT TGTCCAGCAG TTTCACAGCG ATGTCCTTGT  
 40 251 ACCAGTTGGC TGCCTTGCAA GCAGACCTGA TGAACCTGCG CATGGAGCTG  
 301 CAGAGCTACC GAGGTTTCAGC AACACCAGCC GCCGCGGGTG CTCCAGAGTT  
 351 GACCGCTGGA GTCAA ACTCC TGACACCGGC AGCTCCTCGA CCCCACA ACT  
 401 CCAGCCGCGG CCACAGGAAC AGACGCGCTT TCCAGGGACC AGAGGAAACA  
 451 GAACAAGATG TAGACCTCTC AGCTCCTCCT GCACCATGCC TGCCTGGATG  
 45 501 CCGCCATTCT CAACATGATG ATAATGGAAT GAACCTCAGA AACAGAACTT  
 551 ACACATTTGT TCCATGGCTT CTCAGCTTTA AAAGAGGAAA TGCCTTGGAG  
 601 GAGAAAGAGA ACAAATAGT GGTGAGGCAA ACAGGCTATT TCTTCATCTA  
 651 CAGCCAGGTT CTATACACGG ACCCATCTT TGCTATGGGT GATGTCATCC  
 701 AGAGGAAGAA AGTACACGTC TTTGGGGACG AGCTGAGCCT GGTGACCCTG  
 50 751 TTCCGATGTA TTCAGAAATAT GCCCAAACA CTGCCCAACA ATTCTTGCTA  
 801 CTCGGCTGGC ATCGCGAGGC TGGAAAGAAG AGATGAGATT CAGCTTGCAA  
 851 TTCCTCGGGA GAATGCACAG ATTTACGCA ACGGAGACGA CACCTTCTTT  
 901 GGTGCCCTAA AACTGCTGTA ACTCACTTGC TGGAGTGCGT GATCCCCTTC  
 951 CCTCGTCTTC TCTGTACCTC CGAGGGAGAA ACAGACGACT GGAAAACTA  
 55 1001 AAAGATGGGG AAAGCCGTCA GCGAAAGTTT TCTCGTGACC CGTTGAATCT  
 1051 GATCCAAACC AGGAAATATA ACAGACAGCC ACAACCGAAG TGTGCCATGT  
 1101 GAGTTATGAG AAACGGAGCC CGCGCTCAGA AAGACCGGAT GAGGAAGACC  
 1151 GTTTTCTCCA GTCCTTTGCC AACACGCACC GCAACCTTGC TTTTGTGCTT

5  
1201 GGGTGACACA TGTTTCAGAAT GCAGGGAGAT TTCCTTGTTT TGCGATTTGC  
1251 CATGAGAAGA GGGCCCACAA CTGCAGGTCA CTGAAGCATT CACGCTAAGT  
1301 CTCAGGATTT ACTCTCCCTT CTCATGCTAA GTACACACAC GCTCTTTTCC  
1351 AGGTAACTAC TATGGGATAC TATGGAAAGG TTGTTTGTTT TTAAATCTAG  
1401 AAGTCTTGAA CTGGCAATAG ACAAAAATCC TTATAAATTC AAGTGTAATA  
1451 TAAACTTAAT TAAAAAGGTT TAAGTGTG

SEQ. ID NO. 4

10  
1 MDESAKTLP PCLCFCSEKG EDMKVGYPDPI TPQKEEGAWF GICRDGRLLA  
51 ATLLLALLSS SFTAMSLYQL AALQADLMNL RMELQSYRGS ATPAAAGAPE  
101 LTAGVKLLTP AAPRPHNSSR GHRNRRAFQG PEETE QDVDL SAPPAPCLPG  
151 CRHSQHDDNG MNLRNRTYTF VPWLLSFKRG NALEEKENKI VVRQTGYFFI  
201 YSQVLYTDPI FAMGHVIQRK KVHVFGDELS LVTFLFRICIQN MPKTLPNNSC  
15  
251 YSAGIARLEE GDEIQLAIPR ENAQISRNGD DTFFGALKLL

- i. Smith et al. 1990; Kohno et al 1990; Loetscher et al 1990; Schall et al 1990.
- ii. See Jones et al., 1989; Eck et al., 1992.
- iii. K. Tracey, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 255 (1992)); A. Waage, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 275 (1992).
- iv. G. D. Roodman, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 117 (1992).
- v. A. Nakane, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 285 (1992); I. A. Clark et al., in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 303 (1992); G. E. Grau et al., in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 329 (1992); P-F. Piguet, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 341 (1992); G. H. Wong et al., in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 371 (1992).
- vi. S. Malik, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 407 (1992).
- vii. D. A. Fox, Am. J. Med., 99, 82 (1995).
- viii. D. Goeddel et al., Cold Spring Harbor Symposium Quant. Biol., 51, 597 (1986); G. Trinchieri, in Tumor Necrosis Factors. The Molecules and Their Emerging Role in Medicine, B. Beutler (Ed.), Raven Press, NY, p 515 (1992).
- ix. L. A. Tartaglia et al., Proc. Natl. Acad. Sci. USA, 88, 9292 (1991); L.A. Tartaglia and D. V. Goeddel, Immunol. Today, 13, 151 (1992).

- 
- x. B. Luettig et al., J. Immunol., 143, 4034 (1989); M. Kriegler et al., Cell, 53, 45 (1988).
- xi. C. F. Ware et al., in Pathways for Cytolysis, G. M. Griffiths and J. Tschopp (Eds.), Springer-Verlag, Berlin, Heidelberg, p175-218 (1995).
- xii. N. Paul et al., Ann. Rev. Immunol., 6, 407 (1988).
- xiii. P.D. Crowe et al., Science, 264, 707 (1994). (J. Browning et al., Cell, 72, 847 (1993); J. Browning et al., J. Immunol., 154, 33 (1995).
- xiv. P. De Togni et al., Science, 264, 703 (1993); T.A. Banks et al., J. Immunol., 155, 1685 (1995).
- xv. J. Browning and A. Ribolini, J. Immunol., 143, 1859 (1989); J. Browning et al., J. Exp. Med., 183, 867 (1996).
- xvi. T. Suda et al., J. Immunol., 154, 3806 (1995) (T. Suda et al., J. Immunol., 154, 3806 (1995)).
- xvii. B.C. Trauth et al., Science, 245, 301 (1989); S. Yonehara et al., J. Exp. Med., 169, 1747 (1989); S. Nagata and P. Goldstein, Science, 267, 1449 (1995); M. H. Falk et al., Blood, 79, 3300 (1992).
- xviii. F. Rieux-Laucat et al., Science, 268, 1347 (1995); T. Takahashi et al., Cell, 76, 969 (1994); R. Watanabe-Fukunaga et al., Nature, 356, 314 (1992).
- xix. P. R. Galle et al., J. Exp. Med., 182, 1223 (1995).
- xx. F. Silvestris et al., Clin. Immunol. Immunopathol., 75, 197 (1995).
- xxi. P.D. Katsikis et al., J. Exp. Med., 181, 2029 (1995); A. D. Badley et al., J. Virol., 70, 199 (1996).
- xxii. S. Wiley et al., Immunity, 3, 673 (1995).
- xxiii. J. F. Gauchat et al., FEBS Lett., 315, 259 (1993); S. Funakoshi et al., Blood, 83, 2787 (1994).
- xxiv. R. C. Allen et al., Science, 259, 990 (1993).
- xxv. L. Biancone et al., Kidney-Int., 48, 458 (1995); C. Mohan et al., J. Immunol., 154, 1470 (1995).
- xxvi. J. Ruby et al., Nature Medicine, 1, 437 (1995).
- xxvii. Z. Wang et al., J. Immunol., 155, 3722 (1995); A. M. Cleary et al., J. Immunol., 155, 3329 (1995).

- 
- xxviii. S. Hess and H. Engelman, J. Exp. Med., 183, 159 (1996).
- xxix. R. G. Goodwin et al, Cell, 73, 447 (1993); Goodwin et al, Eur. J. Immunol., 23, 2631 (1993); C. A. Smith et al., Cell, 73, 1349 (1993).
- xxx. See for example, Narang, SA (1983) Tetrahedron 39:3; Itakura et al. (1981) Recombinant DNA, Proc 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acid Res. 11:477.
- xxxi. See, for example, Scott et al. (1990) Science 249:386-390; Roberts et al. (1992) PNAS 89:2429-2433; Devlin et al. (1990) Science 249: 404-406; Cwirla et al. (1990) PNAS 87: 6378-6382; as well as U.S. Patents Nos. 5,223,409, 5,198,346, and 5,096,815.
33. M.T. Abreu-Martin, A. Vidrich, D.H. Lynch and S.R. Targan. Divergent induction of apoptosis and IL-8 secretion in HT-29 cells in response to TNF- $\alpha$  and ligation of Fas ligand. J. Immunol. 155: 4147-4154, 1995.
34. K. Agematsu, T. Kobata, F.-C. Yang, T. Nakazawa, K. Fukushima, M. Kitahara, T. Mori, K. Sugita, C. Morimoto and A. Komiyama. CD27/CD70 interaction directly drives B cell IgG and IgM synthesis. Eur. J. Immunol. 25: 2825-2829, 1995.
35. R. Amakawa, A. Hakem, T.M. Kundig, T. Matsuyama, J.J.L. Simard, E. Timms, A. Wakeham, H.-W. Mittrucker, H. Griesser, H. Takimoto, R. Schmits, A. Shahinian, P.S. Ohashi, J.M. Penninger and T.W. Mak. Impaired negative selection of T cells in Hodgkin's disease antigen CD30-deficient mice. Cell 84: 551-562, 1996.
36. J.-L. Bodmer, K. Burens, P. Schneider, K. Hofmann, V. Steiner, M. Thome, T. Bornand, M. Hahne, M. Schroeter, K. Becker, A. Wilson, L.E. French, J.L. Browning, H.R. MacDonald, and J. Tschopp. TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis factor receptor 1 and fas (apo-1/CD95). Immunity 6: 79-88, 1997.
37. J. Brojatsch, J. Naughton, M.M. Rolls, K. Zingler and J.A.T. Young. Carl, a TNFR-related protein is a cellular receptor for cytopathic avian leukemia-sarcoma viruses and mediates apoptosis. Cell 87: 845-855, 1996.
38. J.L. Browning, M.J. Androlewicz and C.F. Ware. Lymphotoxin and an associated 33-kDa glycoprotein are expressed on the surface of an activated human T cell hybridoma. J. Immunol. 147: 1230-7, 1991.
39. J.L. Browning, K. Miatkowski, D.A. Griffiths, P.R. Bourdon, C. Hession, C.M. Ambrose and W. Meier. Preparation and characterization of soluble recombinant heterotrimeric complexes of human lymphotoxins alpha and beta. J. Biol. Chem. 271: 8618-26, 1996.

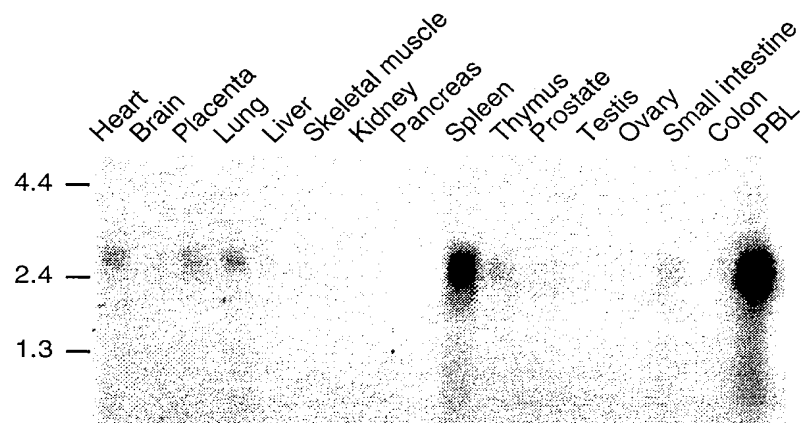
- 
40. J.E. Castro, J.A. Listman, B.A. Jacobson, Y. Wang, P.A. Lopez, S. Ju, P.W. Finn and D.L. Perkins. Fas Modulation of apoptosis during negative selection of thymocytes. Immunity 5: 617-627, 1996.
  41. C.-Y.A. Chen and A.-B. Shyu. AU-rich elements: characterization and importance in mRNA degradation. Trends in Biol. Sci. 20: 465-470, 1995.
  42. Y. Chicheportiche, C. Ody and P. Vassalli. Identification in mouse macrophages of a new 4 kb mRNA present in hematopoietic tissue which shares a short nucleotide sequence with erythropoietin mRNA. Biochim. Biophys. Res. Comm. 209: 1076-1081, 1995.
  43. A.M. Chinnaiyan, K. O'Rourke, G.-L. Yu, R.H. Lyons, M. Garg, D.R. Duan, L. Xing, R. Gentz, J. Ni and V.M. Dixit. Signal transduction by DR3 a death-domain-containing receptor related to TNFR-1 and CD95. Science 274: 990-992, 1996.
  44. P. DeTogni et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science 264: 703-7, 1994.
  45. M.A. DeBenedette, N.R. Chu, K.E. Pollok, J. Hurtako, W.F. Wade, B.S. Kwon and T.H. Watts. Role of 4-1BB ligand in costimulation of T lymphocyte growth and its upregulation on M12 B lymphomas by cAMP. J. Exp. Med. 181: 985-992, 1995.
  46. M. Degli-Esposti, T. Davis-Smith, W.S. Din, P.J. Smolak, R.G. Goodwin and C.A. Smith. Activation of the lymphotoxin- $\beta$  receptor by cross-linking induces chemokine production and growth arrest in A375 melanoma cells. J. Immunol. 158: 1756-1762, 1997.
  47. T.M. Foy, A. Aruffo, J. Bajorath, J.E. Buhlmann and R.J. Noelle. Immune regulation by CD40 and its ligand gp39. Ann. Rev. Immunol. 14: 591-617, 1996.
  48. H.J. Gruss, N. Boiani, D.E. Williams, R.J. Armitage, C.A. Smith and R.G. Goodwin. Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines. Blood 83: 2045-56, 1994.
  49. H.J. Gruss and S.K. Dower. Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. Blood 85: 3378-404, 1995.
  50. J. Kitson, T. Raven, Y.-P. Jiang, D.V. Goeddel, K.M. Giles, K.-T. Pun, C.J. Grinham, R. Brown and S.N. Farrow. A death domain-containing receptor that mediates apoptosis. Nature 384: 372-375, 1996.
  51. S.Y. Lee, C.G. Park and Y. Choi. T cell receptor-dependent cell death of T cell hybridomas mediated by the CD30 cytoplasmic domain in association with tumor necrosis factor receptor-associated factors. J. Exp. Med. 183: 669-674, 1996.

- 
52. R.I. Montgomery, M.S. Warner, B.J. Lum and P.G. Spear. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell 87: 427-436, 1996.
  53. S. Nagata. Apoptosis by death factor. Cell 88: 355-365, 1997.
  54. R.M. Pitti, S.A. Marsters, S. Ruppert, C.J. Donahue, A. Moore and A. Ashkenazi. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J. Biol. Chem. 1996.
  55. C.A. Smith, T. Farrah and R.G. Goodwin. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 76: 959-62, 1994.
  56. G.L. Smith. Virus strategies for evasion of the host response to infection. Trends in Microbiol. 3: 81-88, 1994.
  57. E.Strueber and W. Strober. The T cell-B cell interaction via OX40-OX40L is necessary for the T cell independent humoral immune response. J. Exp. Med. 183: 979-989, 1996.
  58. H.-K. Sytwu, R.S. Liblau and H.O. McDevitt. The roles of Fas/Apo-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. Immunity 5: 17-30, 1996.
  59. P. Vassalli. The pathophysiology of tumor necrosis factors. Ann. Rev. Immunol. 10: 411-452, 1992.
  60. L. Zheng, G. Fisher, R.E. Miller, J. Peschon, D.H. Lynch and M.J. Lenardo. Induction of apoptosis in mature T cells by tumour necrosis factor. Nature 377: 348-351, 1995.





FIG. 2

Fig. Northern Blot  
Kay-L